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Risk Factors Associated with Liver Injury and Impact of Liver Injury on Transplantation-Related Mortality in Pediatric Recipients of Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

In adults, hepatic complications after allogeneic hematopoietic stem cell transplantation (allo-HSCT) are associated with significant morbidity and transplantation-related mortality (TRM). However, there is a paucity of parallel data on the incidence of, and risk factors for, liver injury (LI) and the impact of LI on TRM in pediatric allo-HSCT recipients. We compared total bilirubin, direct bilirubin, and alanine aminotransferase values before allo-HSCT and at 1 month, day +100, and 12 months after allo-HSCT in 248 patients who received either a myeloablative conditioning (MAC) regimen (n = 109) or a reduced-toxicity/reduced-intensity conditioning (RTC/RIC) regimen (n = 139). LI was defined as grade ≥2 hyperbilirubinemia according to the National Cancer Institute's Common Terminology Criteria for Adverse Events 3.0/4.0 (total bilirubin, >1.95 mg/dL, 1.5 times above the upper limit of normal for our laboratory). Univariate and multivariate logistic regression models were used to identify risk factors for LI and TRM. The incidence of LI at 1 month after allo-HSCT was 14.1%. The median bilirubin level was 3.5 mg/dL (range, 1.97 to 32.2 mg/dL). Only LI as defined by total bilirubin level, but not by direct bilirubin or alanine aminotransferase level, was found to be a significant predictor for TRM. The 1-year TRM was 60.7% (95% confidence interval, 42.6% to 78.7%) in patients with LI at 1 month after allo-HSCT, compared with 14.6% (95% confidence interval, 9.9% to 19.4%) ($P < .0001$) in patients those who did not have liver injury. Multivariate analysis identified age ($P = .03$), total body irradiation ($P = .007$), bacterial bloodstream infection (BBSI) ($P = .001$), and invasive fungal infection (IFI) ($P = .002$) as significant risk factors for developing LI at 1 month. On multivariate analysis for risk factors for TRM, only LI at 1 month after allo-HSCT ($P < .0001$), primary graft failure ($P = .001$), BBSI ($P = .003$), and systemic viral infection ($P = .04$) were identified as significant risk factors for TRM. LI before allo-HSCT conditioning was not associated with higher TRM. Although the incidence of LI in pediatric allo-HSCT recipients is low, LI is associated with very high TRM. BBSI and IFI are the primary risk factors for LI.

INTRODUCTION

Hepatic complications affect nearly 80% of adult recipients of allogeneic hematopoietic stem cell transplantation (allo-HSCT) and are associated with significant morbidity and mortality—responsible for up to 15% of transplantation-related mortality (TRM) [1-5]. Previous studies have attempted to determine how best to define liver injury (LI), incorporating clinical, radiologic, and laboratory findings [6]. The extent of LI has been deemed more important than its cause, an important finding given the difficulty of discerning causes of LI in these complex patients [7,8]. Whereas aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin are most commonly used in current clinical practice [6], which of the myriad liver function test abnormalities is truly predictive of TRM remains unclear.

Importantly, the causes of LI before and after allo-HSCT differ between children and adults. In children, the risk of adenoviral infection is much higher, whereas adults are at greater risk of LI secondary to hepatitis B and C infections, alcoholic injury, and other comorbidities. Over the past decade, significant decreases in the incidence of LI and hepatic graft-versus-host disease (GVHD) and subsequent improvements in long-term survival have been linked to less-toxic conditioning regimens and the use of ursodeoxycholic acid for liver protection [9].

Previous studies have demonstrated an association of LI with poor outcome after allo-HSCT [5,8]; however, those reports focused mainly on adult patients and on LI developing after allo-HSCT. There is a paucity of data on the incidence of LI in children after allo-HSCT, as well as data comparing the incidence and impact of LI at various time points and with different conditioning regimens. Consequently, we sought to study the incidence of LI before and after allo-HSCT, to identify risk factors for LI, and to compare the association between LI and TRM in pediatric allo-HSCT recipients who received myeloablative conditioning (MAC) versus those who received reduced-intensity (RIC)/reduced-toxicity conditioning (RTC). Our hypothesis was that MAC would be associated with a higher rate of LI and, subsequently, a higher incidence of TRM.

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METHODS

The patients included in this retrospective analysis were consecutive children, adolescents, and young adults who underwent RIC/RTC or MAC allo-HSCT at New York-Presbyterian Morgan Stanley Children's Hospital between April 2000 and September 2011. Indications for allo-HSCT included a variety of malignant and nonmalignant conditions. Allogeneic sources of stem cells included bone marrow, peripheral blood stem cells, and umbilical cord blood. This retrospective study was approved by Columbia University Medical Center's Institutional Review Board.

Conditioning Regimens

The MAC regimens (n = 105) included total body irradiation (TBI) + cyclophosphamide (120 mg/kg) ± thiotepa (10 mg/kg), TBI + melphalan (90-135 mg/m^2) and 2 alkylators, i.v. busulfan (12.8-16 mg/kg) + cyclophosphamide (120-200 mg/kg), or i.v. busulfan (12.8-16 mg/kg) + melphalan (135 mg/m^2). Twelve patients received a MAC regimen that included gemtuzumab ozogamicin, in addition to i.v. busulfan and cyclophosphamide, as described previously [10]. The RTC regimens (n = 63) included fludarabine (150-180 mg/m^2) + i.v. busulfan (12.8-16 mg/kg) ± alemtuzumab, and fludarabine + cyclophosphamide (200 mg/kg) ± rabbit antithymocyte globulin (8 mg/kg). The RIC regimens (n = 66) included fludarabine + i.v. busulfan (6.4-8 mg/kg) and fludarabine (150 mg/m^2) + cyclophosphamide (60 mg/kg).

GVHD Prophylaxis and Grading

Details on GVHD prophylaxis and grading have been provided previously [11].

Infection Prophylaxis and Supportive Care

All patients were hospitalized in protective isolation, defined as a single hospital room with a high-efficiency particulate air filtration system and reverse isolation. All patients received sargramostim (250 μg/m^2/day) or filgrastim (10 μg/kg/day) either i.v. or s.c. until an absolute neutrophil count >2500/mm^3 was achieved for 3 days, as described previously [12]. Herpes simplex virus prophylaxis consisted of acyclovir (250 mg/m^2) i.v. every 8 hours from day -5 until engraftment and grade II mucositis. *Pneumocystis carinii* prophylaxis consisted of trimethoprim/sulfamethoxazole up to day -2, and then resumed 3 times weekly after myeloid engraftment. Patients unable to tolerate trimethoprim/sulfamethoxazole received i.v. pentamidine prophylaxis every 2 weeks. Fungal prophylaxis consisted of liposomal amphotericin B (3 mg/kg/day i.v.) starting on day 0 and continuing through day +100, as described previously [13]. Cytomegalovirus (CMV) prophylaxis was administered as described previously [14]. After achieving an absolute neutrophil count of >750/mm^3 after allo-HSCT, patients at increased risk of acquiring CMV infection (CMV-positive donor and/or recipient) received prophylaxis with foscarnet (90 mg/kg/dose) every other day alternating with ganciclovir (5 mg/kg/dose) every other day until day +100.

LI Prophylaxis

From 2007 onward, patients were uniformly given ursodiol 30 mg/kg/day for liver protection, starting at 1-7 days before conditioning and continuing up to day +30 after allo-HSCT as tolerated. Patients considered at high risk for veno-occlusive disease (VOD) by the treating physician received prophylaxis with enoxaparin 1 mg/kg s.c. (maximum, 40 mg) from the start of conditioning through day +21 after allo-HSCT [15].

Liver Function Monitoring

In all patients, serum total bilirubin, direct bilirubin, and ALT were measured prospectively 3-4 times per week in the inpatient unit, typically between the start of conditioning and at least 30 days after allo-HSCT. Most of the patients who were discharged had values measured twice a week from day +30 to day +100.

Maximum total bilirubin, direct bilirubin, and ALT values were measured across 2 pre–allo-HSCT time blocks: historic (>2 weeks before the start of conditioning or >1 month before hematopoietic stem cell infusion) and preconditioning (within 2 weeks before the start of conditioning, or approximately day -30 to day -15). Post–allo-HSCT values were collected on an average of 3 days at 1 month (average, day +28 to day +30), 100 days (average, day +98 to day +100), and 1 year after allo-HSCT. For outpatient recipients, data was collected as a single value at each time point. The pre–allo-HSCT blocks were chosen to distinguish between historic and preconditioning LI and its effects on TRM.

Definitions

TRM was defined as death due to any cause other than disease progression or relapse. TRM was measured from the date of allo-HSCT to the date of death or December 31, 2011. Patients were censored at the time of conditioning for a second allo-HSCT or, if they remained alive, on December 31, 2011.

Poor-risk patients were defined as those with chemoresistant malignant disease, third or greater complete remission, induction failure, progressive disease, and/or receipt of a second allograft. All other patients with malignant or nonmalignant diseases were defined as average risk.

LI was defined as total bilirubin >1.95 mg/dL, which is grade 2 hyperbilirubinemia as defined by the National Cancer Institute's Common Terminology Criteria for Adverse Events 3.0/4.0 (specifically, >1.5 times the upper limit of normal for our laboratory; the normal total bilirubin value in our laboratory is 0.3 mg/dL to 1.3 mg/dL).

Of note, although data on direct bilirubin and ALT were collected initially, these variables were not significant predictors of TRM in our multivariate analysis, and so we limited our analysis to total bilirubin.

Acute kidney injury was defined as a >50% drop in estimated creatinine clearance compared with baseline as evaluated using the formula of Schwartz [11].

Invasive fungal infections (IFIs) were divided into candidemia, invasive aspergillosis, and other fungi and defined using previously published criteria [16]. Systemic viral infection (SVI) was defined as a positive blood PCR finding or as we have previously published [16]. Bacterial bloodstream infection (BBSI) was defined as a positive blood culture for gram-positive cocci, gram-negative rods, and atypical mycobacterium.

Statistical Methods

Values for continuous variables are presented as mean ± standard deviation, and values for categorical variables are presented as number and percentage. Between-group comparisons were made using the *t*-test for continuous variables and the chi-square test for categorical variables. Univariate and multivariate logistic regression models were used to identify risk factors for LI, and the competing-risk regression analysis of Fine and Gray [17] was used for TRM, with relapse as the competing event. Multivariate analysis was performed using factors that had a P value <.20 on univariate regression analysis, using a stepwise variable selection procedure with .1 as the entry level and .05 as the stay level. A P value <.05 was considered to indicate statistical significance. All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC).

Risk factors for LI analyzed included age, sex, year of allo-HSCT, CMV risk status, disease type (malignant versus nonmalignant), disease risk status, SVI, BBSI, IFI, acute kidney injury, stem cell source (matched sibling donor/matched unrelated donor versus umbilical cord blood), conditioning (MAC versus RTC/RIC), primary graft failure, TBI, enoxaparin prophylaxis, ursodiol prophylaxis, CD34 selection, and aGVHD prophylaxis with methotrexate. Risk factors for TRM analyzed included age, sex, year of allo-HSCT, CMV risk status, disease type and disease risk status, acute and chronic GVHD, VOD, conditioning regimen, primary graft failure, stem cell source, IFI, BBSI, SVI, historic and preconditioning LI, and LI at 1 month after allo-HSCT. The analyses were performed using SAS 9.2 and R with the cmprsk package [18].

RESULTS

Patients and Demographics

A total of 248 eligible patients (164 males and 84 females; mean age, 9.54 ± 6.41 years) underwent allo-HSCT during the study period. The MAC group comprised 109 patients (mean age, 8.98 ± 6.07 years), of whom 87 (79.8%) underwent allo-HSCT for malignant disorders and 22 (20.2%) did so for nonmalignant disorders. The RTC/RIC group comprised 139 patients (mean age, 9.99 ± 6.66 years), of whom 66 (47.5%) underwent allo-HSCT for malignant disorders and 73 (52.5%) did so for nonmalignant disorders. Data were initially collected and analyzed independently for the RTC and RIC groups; however, because the incidence of LI and risk factors for LI and TRM were nearly identical in these 2 groups, with no statistically significant differences, these 2 groups were combined and reanalyzed.

Pre–allo-HSCT demographic data for all patients are displayed in Table 1. There was no statistically signficant differences between MAC allo-HSCT recipients and RTC/RIC allo-HSCT recipients in terms of age, sex, body mass index, CMV risk status, or donor source. However, more children in the MAC allo-HSCT group underwent transplantation for

Table 1
Pretransplantation Demographic Data for Pediatric Allo-HSCT Recipients Conditioned with RTC/RIC and with MAC

Characteristic	All (n = 248)	MAC (n = 109)	RTC/RIC (n = 139)	*P* Value
Age, yr, mean ± SD	9.5 ± 6.4	9.0 ± 6.1	10.0 ± 6.7	.22
Sex, n (%)				
Male	164 (66)	67 (61)	97 (70)	.17
Female	84 (34)	42 (38)	42 (30)	
Disease type, n (%)				
Malignant	153 (62)	87 (80)	66 (47)	<.001
Nonmalignant	95 (38)	22 (20)	73 (52)	
Disease status, n (%)				
Average-risk group	187 (75)	69 (63)	118 (85)	<.001
Poor-risk group	61 (25)	40 (37)	21 (15)	
CMV risk status, n (%)				
Donor and/or recipient seropositive	178 (72)	84 (77)	94 (68)	.10
Donor and recipient seronegative	70 (28)	25 (23)	45 (32)	
Donor, n (%)				
Matched sibling donor	89 (36)	35 (32)	54 (39)	.46
Matched unrelated donor	51 (21)	27 (25)	24 (17)	
Related cord blood	6 (2)	3 (3)	3 (2)	
Unrelated cord blood	102 (41)	44 (40)	58 (42)	

a malignant condition ($P < .0001$), and more had poor-risk disease ($P < .0001$).

Post–allo-HSCT characteristics, not including LI, are presented in Table 2. There were no statistically significant differences in the incidence rates of SVI, IFI, chronic GVHD, primary graft failure, or VOD between the MAC allo-HSCT and RTC/RIC allo-HSCT groups. The incidence rates of BBSI ($P = .036$) and acute GVHD ($P < .0001$) were higher in the MAC allo-HSCT group.

Table 2
Post-transplantation Outcomes of Pediatric Allo-HSCT Recipients Conditioned with RTC/RIC and with MAC

Characteristic	All (n = 248)	MAC	RTC/RIC	*P* Value*
SVI (0-30 d)	(n = 246)			
No	215 (87)	95 (87)	120 (88)	.92
Yes	31 (13)	14 (13)	17 (12)	
BBSI (0-30 d)	(n = 247)			
No	188 (76)	76 (70)	112 (81)	.04
Yes	59 (24)	33 (30)	26 (19)	
IFI (0-30 d)	(n = 247)			
No	240 (97)	105 (96)	135 (98)	.48
Yes	7 (3)	4 (4)	3 (2)	
Primary graft failure	(n = 243)			
No	214 (88)	97 (91)	117 (85)	.14
Yes	29 (12)	9 (9)	20 (15)	
Acute GVHD	(n = 248)			
No	148 (60)	50 (46)	98 (70)	<.0001
Yes	100 (40)	59 (54)	41 (30)	
Chronic GVHD	(n = 248)			
No	203 (82)	89 (82)	114 (82)	.94
Yes	45 (18)	20 (18)	25 (18)	
VOD	(n = 248)			
No	230 (93)	98 (90)	132 (95)	.13
Yes	18 (7)	11 (10)	7 (5)	
Enoxaparin	(n = 247)			
No	145 (59)	49 (45)	96 (69)	.0002
Yes	102 (41)	59 (55)	43 (31)	
Ursodiol	(n = 247)			
No	155 (63)	62 (57)	93 (67)	.13
Yes	92 (37)	46 (43)	46 (33)	
Methotrexate	(n = 248)			
No	212 (86)	88 (81)	124 (89)	.06
Yes	36 (14)	21 (19)	15 (11)	

All data are n (%).
* Chi-square test.

LI

Pre–Allo-HSCT

Historic and preconditioning values for total bilirubin for patients with these data available are presented in Table 3. Thirty-three patients with a hemoglobinopathy, specifically sickle cell disease or thalassemia, were excluded from this pre–allo-HSCT bilirubin analysis, and another 5 patients who were referred from other institutions for allo-HSCT did not have historical bilirubin values available. There was no statistically significant difference in historic or preconditioning LI between the MAC allo-HSCT and RTC/RIC allo-HSCT groups.

Post–Allo-HSCT

The incidence of LI at 1 month, 100 days, and 1 year after allo-HSCT are listed in Table 3. By 1 month after allo-HSCT, 33 patients (14.1%) had developed LI. The incidence of LI at 1 month was 21.9% in the MAC allo-HSCT group versus 7.8% in the RTC/RIC allo-HSCT group ($P = .0067$). In the patients with LI at 1 month after allo-HSCT, the median total bilirubin level was 3.5 mg/dL (range, 1.97 to 32.2 mg/dL). There was no statistically significant differences in LI at day +100 or 1 year after allo-HSCT between the MAC and RTC/RIC groups.

Risk Factors for Developing LI at 1 Month

Because the incidence of LI after allo-HSCT was highest at 1 month and was substantially decreased by day +100, we analyzed risk factors for LI at 1 month. On univariate analysis, the risk factors for developing LI at 1 month after allo-HSCT with a *P* value <.20 were age ($P = .10$), year of allo-HSCT ($P = .12$), disease risk ($P = .0016$), BBSI ($P < .0001$), IFI ($P = .0013$), acute kidney injury ($P = .0056$), stem cell source ($P = .09$), MAC ($P = .0029$), TBI ($P = .002$), and aGVHD prophylaxis with methotrexate ($P = .11$). Sex, CMV risk status, malignant disease, SVI, enoxaparin prophylaxis, ursodiol prophylaxis, and primary graft failure were not significant.

Table 3
Incidence of Pretransplantation and Post-transplantation LI, Defined by Total Bilirubin Concentration, in Pediatric Allo-HSCT Recipients Conditioned with RTC/RIC and with MAC

Total Bilirubin, mg/dL*	All (n = 248)	MAC	RTC/RIC	*P* Value†
Past (>1 mo before allo-SCT)	(n = 177)			
≤1.3	102 (58)	52 (62)	50 (54)	.51
1.3-1.95	24 (14)	11 (13)	13 (14)	
>1.95	51 (29)	21 (25)	30 (32)	
Preconditioning (d -30 to d -15)	(n = 206)			
≤1.3	186 (90)	88 (93)	98 (89)	
1.3-1.95	13 (6)	6 (6)	7 (6)	
>1.95	7 (4)	1 (1)	6 (5)	.23
1 mo after allo-SCT	(n = 234)			
≤1.3	189 (81)	76 (72)	113 (87)	.007
1.3-1.95	12 (5)	6 (6)	6 (5)	
>1.95	33 (14)	23 (22)	10 (8)	
Day +100 after allo-SCT	(n = 205)			
≤1.3	190 (93)	75 (89)	115 (95)	.10
1.3-1.95	9 (4)	4 (5)	5 (4)	
>1.95	6 (3)	5 (6)	1 (1)	
1 yr after allo-SCT	(n = 128)			
≤1.3	124 (97)	43 (96)	81 (98)	.39
1.3-1.95	1 (1)	0 (0)	1 (1)	
>1.95	3 (2)	2 (4)	1 (1)	

Data are n (%).
* Missing patients were referred from other institutions or underwent allo-HSCT promptly after diagnosis.
† Chi-square test.

Table 4
Multivariate Analysis for Risk Factors Associated with LI at 1 Month in Pediatric Allo-HSCT Recipients

Variable	OR	95% CI	*P* Value
Age	1.1	1.0-1.2	.03
BBSI			
No	1		
Yes	5.0	1.9-13.1	.001
IFI			
No	1		
Yes	44.4	4.0-497.4	.002
TBI			
No	1		
Yes	4.0	1.5-11.1	.007

On multivariate analysis, older age ($P = .03$), BBSI ($P = .001$), IFI ($P = .002$), and TBI ($P = .007$) were significant risk factors for developing LI at 1 month (Table 4).

BBSI and IFI

Because BBSI and IFI during the first 30 days after allo-HSCT were highly associated with LI at 1 month, we analyzed causative organisms in more depth. During the first 30 days after allo-HSCT, 18 of the 33 patients experienced a total of 28 episodes of BBSI (12 gram-negative rods, 10 *Staphylococcus* spp, 3 *Streptococcus* spp, and 3 vancomycin-resistant enterococci). Eight IFIs were noted in 6 patients (5 *Candida* spp, 1 *Aspergillus niger*, 1 *Malassezia furfur*, and 1 *Cryptococcus neoformans*).

TRM

LI and various other transplantation-related factors can lead to mortality in patients after allo-HSCT. The incidence of TRM during the study period of 1095 ± 1173.3 days (range, 15-4258 days) was higher in the MAC group compared with the RTC/RIC group (33.9% [37 of 109] versus 18.1% [25 of 138]; $P = .004$). We performed univariate proportional competing-risk regression analysis for risk factors associated with TRM after RTC/RIC allo-HSCT and MAC allo-HSCT. In terms of LI, historic total bilirubin >1.95 mg/dL, direct bilirubin >0.98 mg/dL, and ALT >200 U/mL were not statistically significant, nor was preconditioning total bilirubin >1.95 mg/dL. Total bilirubin >1.95 mg/dL at 1 month ($P < .0001$), MAC conditioning ($P = .005$), primary graft failure ($P < .0001$), stem cell source ($P = .02$), SVI ($P = .039$), BBSI ($P < .0001$), VOD ($P = .0047$), and IFI ($P = .001$) were all statistically significant on univariate analysis. Allo-HSCT performed between 2006 and 2011 had a trend toward lower TRM ($P = .067$). Age, sex, CMV risk status, disease type, disease risk status, acute GVHD, and chronic GVHD were not statistically significant.

On multivariate competing-risk regression analysis, only total bilirubin >1.95 mg/dL at 1 month ($P < .0001$), primary graft failure ($P = .001$), SVI ($P = .04$), and BBSI ($P = .003$) remained significant predictors of TRM (Table 5). Of note, MAC conditioning was no longer a significant risk factor for TRM on multivariate analysis.

Table 5
Multivariate Competing-Risk Regression on TRM after RIC/RTC versus MAC in Pediatric Allo-HSCT Recipients

Variable	OR	95% CI	*P* Value
Primary graft failure			
No	1		
Yes	2.8	1.5-5.3	.001
SVI			
No	1		
Yes	2.2	1.0-4.5	.04
BBSI			
No	1		
Yes	2.4	1.3-4.2	.003
Total bilirubin (day 30)			
≤1.95 mg/dL	1		
>1.95 mg/dL	7.9	4.4-14.2	<.0001

LI and TRM

Twenty-three of the 33 patients with a total bilirubin >1.95 mg/dL at 1 month after allo-HSCT sustained TRM. Further analysis of causes of death in these patients revealed that the majority were related to infection. Specifically, 14 of the 23 patients who died had a severe bacterial infection, 3 had a fungal infection, 2 had a viral infection, and 1 had toxoplasmosis. In addition, 4 patients had VOD, and 1 patient died secondary to intracranial hemorrhage from moyamoya disease. Figure 1 shows the impact of LI at 1 month on TRM. The 1-year probability of TRM was significantly greater in patients with LI at 1 month compared with those who did not (60.7% [95% CI, 42.6% to 78.7%] versus 14.6% [95% CI, 9.9% to 19.4%]; $P < .0001$).

DISCUSSION

The present study has examined the incidence and impact of LI occurring before and after allo-HSCT in pediatric recipients. To our knowledge, this is the largest study of hepatic dysfunction and subsequent outcomes in pediatric allo-HSCT recipients reported to date.

We used the National Cancer Institute's Common Terminology Criteria for Adverse Events 3.0/4.0 to define LI as laboratory values indicative of grade ≥2 hyperbilirubinemia. Similar to other studies [6], we found that total bilirubin, but not ALT, was useful in predicting mortality after allo-HSCT. Furthermore, pre–allo-HSCT LI did not predict post–allo-HSCT TRM.

Few previous studies have focused on pre–allo-HSCT LI. Barba et al. [6] reported that in adult allo-HSCT recipients, pretransplantation elevations of bilirubin and γ-glutamyl-transpeptidase, but not of AST, ALT, or alkaline phosphatase,

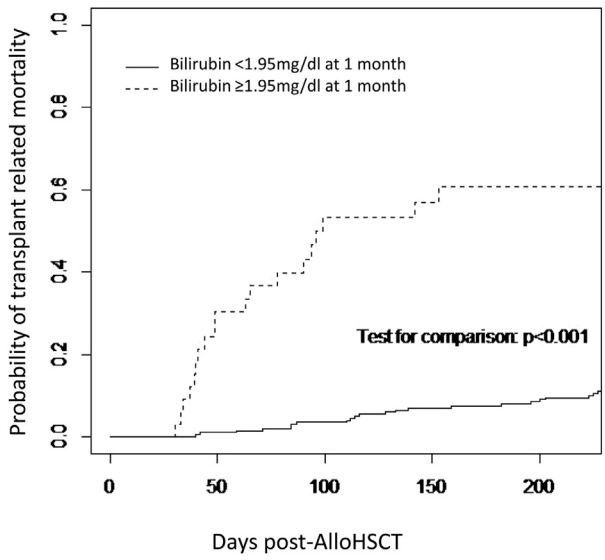

Figure 1. TRM stratified by presence of LI at 1 month.

resulted in higher day +100 TRM and lower overall survival. In the present study, we were unable to demonstrate the impact of pre–allo-HSCT LI on TRM in pediatric patients, possibly because of our small study cohort or the fact that patients with pre–allo-HSCT LI might have been more likely to receive RTC/RIC. Indeed, 14 of our 20 patients with pre–allo-HSCT LI received RTC/RIC. Confirming this finding in a larger prospective study will be important, considering that pretransplantation ALT and bilirubin levels are eligibility criteria for allo-HSCT, and transplantation is often delayed until these laboratory values normalize.

In the present study, 33 patients (14.1%) had LI, defined as ≥grade 2 hyperbilirubinemia, by 1 month after allo-HSCT. The incidence of LI in our pediatric cohort was lower than that reported previously in adult allo-HSCT recipients [19,20]. In a study of 193 adult recipients of allo-HSCT with nonmyeloablative conditioning, Hogan et al. [19] reported severe hyperbilirubinemia (>4 mg/dL) in 24% and some degree of hyperbilirubinemia (total bilirubin >1.2 mg/dL) in 84%. Similarly, Kusumi et al. [20] found that 87% of adult RIC unrelated cord blood transplant recipients had a total serum bilirubin level >1.2 mg/dL. In the present study, only 19.2% of our pediatric patients had a total serum bilirubin level >1.2 mg/dL (data not shown). Furthermore, the incidence of post-transplantation LI declined steeply over time, with rates of 2.9% at day +100 and only 2.3% at 1 year. This decrease in LI is likely secondary to the high mortality rate associated with LI at 1 month after allo-HSCT.

The 1-year TRM in our patients with LI at 1 month after allo-HSCT was extremely high, at 60.7%. Furthermore, the patients with TRM died early, by day +200 after allo-HSCT. In an adult study, allo-HSCT recipients with extreme hyperbilirubinemia (>10 mg/dL) had a day +200 TRM of 79%, compared with 17% in those with total bilirubin <10 mg/dL [8]. In the present study, the patients with LI at 1 month who subsequently died had a median total bilirubin level of 5.33 mg/dL, and only 20% had extreme hyperbilirubinemia. TRM was 100% in the patients with extreme hyperbilirubinemia. Taken together, our data suggest that pediatric allo-HSCT recipients have a lower incidence of LI, but higher associated TRM, compared with adult recipients.

BBSIs and ISIs were the most significant risk factors for LI in our pediatric allo-HSCT recipients. BBSI has been previously identified as a risk factor for LI [20], but the mechanism for this remains unclear. It is plausible that during the early post–allo-HSCT period, recipients of MAC have higher grades of mucositis, which could lead to transmigration of gastrointestinal bacteria into the bloodstream, resulting in sepsis [21]. The association between bacterial infection during the first 30 days after allo-HSCT and hyperbilirubinemia at 1 month and its impact on subsequent TRM is intriguing. It is plausible that bacterial infections may start the cascade of events that leads to hyperbilirubinemia and ultimately TRM. Prospective studies of the associations among BBSI, LI, and TRM are planned for the next few years.

Patients with IFI who subsequently develop LI likely fall into 2 classes: those whose infection leads directly to LI and, more frequently, those with antifungal-mediated LI. A recent study of the hepatotoxicity of antifungal medications in bone marrow transplant recipients found that both liposomal amphotericin B and fluconazole were associated with a substantial increase in the risk of hepatotoxicity [22]. Further analysis demonstrated that in patients who developed hepatotoxicity and who continued receiving antifungal therapy, marked hyperbilirubinemia developed in 33% of the patients treated with liposomal amphotericin B, compared with only 8% of those treated with fluconazole.

We were interested in the role of conditioning regimen on LI. Previous studies have implicated MAC regimens, TBI, and methotrexate as risk factors for LI [23-26], and each of these factors was significantly associated with LI on univariate analysis. Only TBI-containing conditioning regimens remained significant on multivariate analysis. However, MAC and TBI-containing regimens were not associated with a higher incidence of TRM when controlling for other risk factors.

Previous studies have demonstrated the protective effect of ursodiol and low molecular weight heparin in prophylactic prevention of hepatic injury; however, these findings were not duplicated in the present study. Ruutu et al. [27] reported that fewer adult allo-HSCT recipients receiving ursodiol developed hyperbilirubinemia ($P = .04$) or grade III to IV aGVHD ($P = .01$) compared with those not receiving ursodiol. Similarly, Thornley et al. [28] found that pediatric allo-HSCT recipients receiving prophylactic doses of ursodiol experienced less severe hepatic toxicity ($P = .007$). In the present study, 37.3% of our pediatric allo-HSCT recipients received prophylactic ursodiol, but univariate analysis showed no protective effect (hazard ratio [HR], 1.137; $P = .7270$). Similarly, Simon et al. [29] reported that adult recipients of allo-HSCT receiving prophylactic low molecular weight heparin were at reduced risk of developing VOD ($P = .0005$) compared with those receiving heparin ($P = .002$) or no prophylaxis. In the present study, 41.3% of our patients received enoxaparin, but univariate analysis showed no protective effect (HR, 0.94; $P = .87$), similar to previous reports [15]. The lack of a protective effect of ursodiol on the development of LI may be related to our small sample size, however.

Limitations of this retrospective study include disparate donor sources and disease risk status and lack of uniformity in administration of ursodiol and enoxaparin. Because subjects were heterogeneous in terms of underlying disease, conditioning regimen, and hematopoietic stem cell donor sources, this preliminary analysis should be replicated in additional studies of children with more homogenous diagnoses and cell sources. Another limitation of this study is that we did not investigate causes of LI. LI may precede allo-HSCT, with causes including chronic hepatitis B and C infection, fungal infection, previous chemotherapy, and previous radiation therapy, or may occur after allo-HSCT from the conditioning chemotherapy regimen, VOD, GVHD, administration of total parenteral nutrition, ischemia, drug-induced hepatic injury, or infection [30-32]. We are planning a prospective study of causes of LI in children at our center in an attempt to better identify prophylactic and therapeutic measures to decrease the incidence and improve outcomes for these patients.

In summary, in our study cohort, early LI was strongly associated with early TRM, and BBSI and IFI were the primary risk factors for LI. These findings underscore the crucial need to further minimize bloodstream infections. Various strategies are currently used to decrease bloodstream infections [33]. The National Association of Children's Hospitals and Related Institutions is working to minimize bloodstream infections secondary to catheters and central lines by standardizing procedures for dressing changes and obtaining blood from central lines. In addition, an ongoing study by the Children's Oncology Group is evaluating the toxicity and efficacy of levofloxacin in decreasing bacteremia in pediatric

allo-HSCT recipients. At our institution, as we determine which organisms are able to cause breakthrough infections in patients already receiving ongoing prophylactic antifungal and antibacterial agents, we will seek to develop alternative prophylactic and/or therapeutic regimens. Together, these approaches should serve to further decrease the incidence of LI, and thus TRM, in pediatric allo-HSCT recipients.

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